OBSERVATIONS ON THE ONTOGENY OF SCLEREIDS IN LEAVES OF NYMPHAEA ODORATA

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ABSTRACT

BIOLOGY

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Observations on the Ontogeny of Sclereids in Leaves of Nymphaea odorata

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Sclereids are conspicuous and abundant in leaves of Nymphaea odorata. The ontogeny of these sclereids has been studied in petioles and lamina of leaves at different stages of maturation. Nuclear enlargement of certain cells in the ground tissue of leaf primordia provide the first clear indication of incipient sclereid differentiation. Further development involves increased vacuolation, outgrowth of protuberances, deposition of secondary wall layers, and eventually complete loss of the protoplast. In young petioles, sclereids were observed to originate from cells bordering air canals that became enlarged. In the lamina of leaf primordia, the time of sclereid initiation appears to be correlated with air space formation in the mesophyll. Some variation in sclereid morphology was found when those occurring in the mesophyll regions and petioles were compared. Stellate sclereids were the predominant type in the spongy region, mostly I-shaped sclereids in the palisade, and H-shaped trichosclereids in the petiole.

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CHAPTER I

INTRODUCTION

There have been numerous studies on sclereid ontogeny. Early work on the subject was advanced by some investigators during the nineteenth century. According to Foster (1944), one of these investigators, Cavara, noticed that the enlargement of the cell nucleus was the first indication of a sclereid initial. Since that time, workers used that observation as the basis for their work. Early in the twentieth century, studies by Conard (1905) dealt mainly with a description and distribution of mature sclereids. Other twentieth century investigators such as Arzee (1953a, 1953b), Bloch (1946), Gaudet (1960), Malaviya (1957) and some other investigators have been interested in the problem but most of the chief impetus has been provided by Foster (1944, 1945, 1946, 1949, 1955).

In all reports reviewed, there is a similarity in sclereid ontogenesis but differences in morphology and distribution. In the lamina of leaves, sclereids may be terminal or may be found throughout the lamina except at the margin. In some petioles they may be associated with air canals or scattered throughout the ground tissue. Sclereid morphology varies from long slender forms to stellate forms.

The present work attempts to determine whether or not variations occur in the pattern of sclereid ontogeny in Nymphaea odorata, as reported by Gaudet (1960) and, also, to determine whether or not there are differences

in the morphology of sclereids found in leaves of this plant species. In addition to a corroboration of Gaudet's findings, an attempt is made to determine if sclereids develop in areas other than in association with air canals.

Many variations in sclereid morphology occur. However, characteristic types are found in the family Nymphaceae. Because of these similarities, it is felt that studies on sclereid morphology in the family may shed some light on taxonomic relationships within the group. In addition, it is felt that information learned from this investigation may contribute towards a further understanding of sclereid ontogeny in Nymphaea odorata.

CHAPTER II

REVIEW OF LITERATURE

Although the ontogeny of sclereids among different genera and species of plants show a similar relationship, there are considerable variations in sclereid morphology. Position may contribute to morphological differences. Some sclereids, arising as idioblasts, have distinct morphologic forms whereas others may develop by the sclerosis of parenchyma cells. An idioblast, as defined by Esau (1960), is a cell in a tissue which markedly differs in form, size, or content from other cells in the same tissue. Cells which become sclerotic resemble their neighboring cells. Three types of idioblasts were mentioned by Foster (1946); namely, excretory, tracheiod, and sclerenchymatous. Most attention has been given to the latter because of their polymorphism.

Foster (1946), in his studies on the ontogeny of sclereids in species of Mouriria, was not able to ascertain whether the terminal sclereids originated from procambial cells in the developing veinlets, or from adjacent cells of the ground meristem. These sclereids were found to arise very early. In Mouriria huberi, Foster (1947) reported that in the leaf lamina, sclereids and associated procambial strands arise from a common cell layer. It was noticed that the formation of a sclereid initial in the spongy region is accompanied by longitudinal division of a "procambial mother cell" in direct contact with the initial. Similarly, sclereids in Monstera

deliciosa, were characterized as trichosclereids. They were found to originate from late, differential, polar divisions of cortex cells near the root apex, in a region analogous to the hypodermis. When mature, these sclereids become hair-like in appearance.

Reports on sclereid ontogeny indicate that sclereid initials become greatly enlarged or elongated. Arzee (1953a) reported three types of sclereids in the lamina of Olea europaea. "T" shaped sclereids were found to originate in the palisade parenchyma. The subepidermal layer was found to be made up of elongated branches of sclereids lying on the outer side of the palisade layer. These sclereids resembled fibers. Variously branched sclereids originated in the spongy layer.

Idioblastic sclereids in the lamina of Trochodendron aralioides originate from young spongy parenchyma cells. According to Foster (1945) these sclereids, upon development, eventually extend their tubular branches across prominent air chambers. Observations by Foster (1955), also, indicate that initials in Boronia serrulata arise in the spongy region. None were reported to occur in the palisade region. These initials are not discernable until after the tracheary elements have completed their development in a veinlet ending.

Sclereids are also abundant in the covering of seeds, in the pith and the cortex. Abundant sclereids constitute the outer covering of macrosclereids and osteosclereids in the testa of Pisum sativum (Reeve, 1946). A study on the comparative histogenesis of such sclereids revealed that the macrosclereids develop from a well defined protoderm in the young ovule. The hypodermis does not become differentiated to form osteosclereid initials until the protoderm cells have begun to elongate.

In gymnosperms and dicotyledons, the cortex and pith often contain sclereids. In the variety of Camellia japonica used by Foster (1944), it was observed that sclereids develop from parenchymatous cells of the cortex and pith during the last phase of enlargement of the foliage leaf. Also, in the pith, Sterling (1947) observed sclereids in the shoot of Pseudotsuga taxifolia. They are first noticeable among the elongated pith cells just below the zone where transverse expansion of the pith occurs.

Mia (1964) observed the appearance of sclereids in the stem of Rauwolfia vomitoria. These sclereids occur in the pith, the leaf gap, the leaf base, and differentiate from ground parenchyma. All are concentrated at the nodes.

An account has been given by Conard (1905) of the anatomy of different species in the family Nymphaceae. Sclereid morphology and distribution were mentioned but no reports were made on sclereid ontogeny.

Gaudet (1960) demonstrated that sclereids in leaves of Nymphaea odorata develop both in the petiole and lamina. In the petiole, the cells bordering the air spaces initiate sclereids. Their morphology varies from bipolar to stellate forms. In the leaf lamina, sclereids occur both in the palisade and spongy regions. The sclereids in the palisade region are elongate and send their branches into the spongy region. Stellate sclereids appear in the latter region and branch profusely into the spaces abutting upon each other. The sclereids formed in these leaves exhibit prominent crystals. According to Gaudet (1960), Gurtler and Frey-Wyssling reported that crystals appear between the primary and secondary walls during sclereid maturation.

Sclereid ontogenesis in Nymphoides is very similar to that in Nymphaea odorata. Malaviya (1957) also observed sclereids developing from cells of

the ground tissue lining the air canals in petioles of Nymphoides cristatum. In the lamina, generally, one of the cells of the spongy mesophyll converts into a sclereid. Very rarely a cell from the palisade layer, which is near a vascular bundle, differentiates into a sclereid.

Some of the reports reviewed have provided evidence that sclereids develop from a single cell or from mother cells that divide. The cells vary in size and form depending upon the amount of available space for expansion and branching. In some cases, air space formation is not correlated with sclereid ontogeny. The appearance of vascular bundles, in an organ where sclereids form, appear to be characteristically associated with sclereid ontogenesis.

CHAPTER III

MATERIALS AND METHODS

The material that was used for this investigation was collected from a city park in Atlanta. Young shoots from the rhizome of Nymphaea odorata were collected during the months of April, May, June, and July. Preliminary observations of some free-hand sections of shoots showed that sclereids were numerous. Preliminary sections of leaves were also made. In this material, sclereids appeared to be absent. As a result, this kind of material was utilized for critical studies on sclereid ontogeny. Petioles measuring from 6-18 mm and lamina of leaves ranging from 5-15 mm were used for this investigation.

Leaf material to be studied was first cut into small pieces and then placed in FPA (formalin-proprionic acid - ethyl alcohol), a killing and fixing fluid. Some of the pieces were fixed in CRAF III. Most of the material was fixed in FPA, for when cell fixations in the two fluids were compared, no appreciable difference was noted in the histological character of the structures studied. Subdividing the material into small pieces afforded quick penetration of the fixative. The material was allowed to remain in the fixative for at least 24 hours. Fixed material was prepared for paraffin infiltration by the tertiary-butyl alcohol series. Following dehydration, the material was infiltrated with a low temperature paraffin (50-52C), and embedded in Fisher's tissue mat.

Two staining schedules were followed, namely: a safranin-fast green method (Johansen, 1940) and a tannic acid-iron chloride-safranin method as outlined by Jensen (1962). In safranin and fast green, primary walls were stained green, and walls which were secondarily thickened, were stained red. Nuclei also stained red. When the tannic acid-ferric chloride stain combination was used, primary cell walls stained black and the cytoplasm blue-gray; safranin stained the nucleoli and the lignified cell walls red. In both schedules, the nucleoli stained more intensely than the nucleus.

Longitudinal and cross sections of petioles were prepared for microscopic study. All sections were cut at 10 microns. Paradermal and cross sections of leaves were also sectioned at 10 microns.

Some material was macerated in order to study the morphology of entire sclereids. Small pieces of material for maceration were placed in a vial and aspirated in an acid-alcohol mixture of equal parts of 10% chromic acid and 10% nitric acid. The vial was corked and then placed in an oven at a temperature of 30-40C for about two days or until the material became soft in texture. The macerated material was washed in distilled water to remove as much acid as possible and transferred to 50% alcohol for further study. Pieces of leaf parts were teased apart and placed in a small amount of water containing safranin. Drops of water containing sclereids were removed to slides by pipette, air dried, and mounted in glycerine jelly (Foster, 1949).

In order to study the distribution and pattern of sclereids in the lamina of the leaf, it was necessary to observe cleared leaves. Small leaves were cleared in toto, and larger leaves were divided into right and left halves. The material was then placed in bowls containing 5% aqueous NaOH solution. Frequent changes were not necessary because, being young, the

material became devoid of coloration earlier than if it had been older. The time required for the material to become translucent was from 4 to 5 days. Syracuse watch glasses were used as receptacles to make transfers, using a camel-hair's brush, through the following series: 50% alcohol, 95% alcohol, 100% alcohol (two changes), 100% alcohol-xylene, and pure xylene. Safranin was added to the alcohol before transferring the material to xylene (Foster, 1949). The introduction of safranin to the 100% alcohol-xylene stained the sclereids, and the translucent nature of the attached petiole provided means, also, of observing the morphology of petiolar sclereids.

CHAPTER IV

OBSERVATIONS AND DISCUSSION

Petiolar Sclereids

Petioles in Nymphaea odorata, as in many aquatic plants, have a highly lacunate system as a result of the presence of numerous air-canals. In this species, the petiole is of the type as found in Eu-castalia, consisting of four nearly equal, large canals centrally positioned. These canals are oppositely arranged in sets of two's. Other canals surround these central ones.

Petiolar sclereids originate from certain cells bordering air-canals. In addition, certain cells that are associated with air spaces may likewise become sclereids. The sclereid initials become noticeable after the petiole reaches a length of approximately 6 mm. The leaf lamina at this time is still rolled and is approximately 5 mm in length. The first indication of the initiation of a sclereid is nuclear enlargement of the precursor cell (fig. 1). This is accompanied by a general enlargement and vacuolation of the sclereid initial (figs. 2, 3). The large vacuole appears first, usually in the central portion or near the tip of the initial (fig. 3). Associated with nuclear enlargement and vacuolation of the initial is a change in nuclear position (fig. 4). This relocation of the nucleus involves a shifting from the central region of the sclereid into the expanding arm. This phenomenon of nuclear migration has been noted in other studies on sclereid

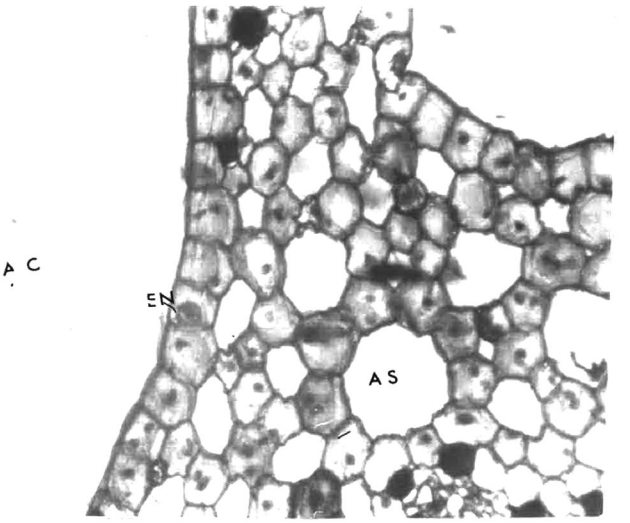

Fig. 1. Transverse section through petiole showing sclereid initial with enlarged nucleus; AC-air-canal, EN-enlarged nucleus, AS-air space. X100.

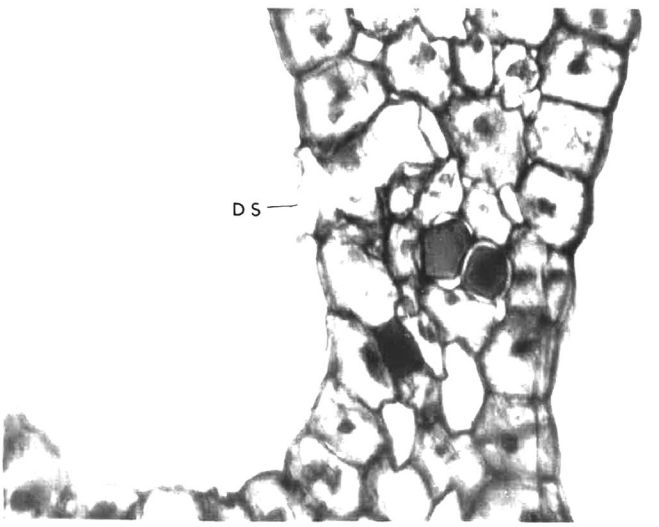

Fig. 2. Transverse section through petiole showing enlarging sclereid initial; DS-developing sclereid initial. X100.

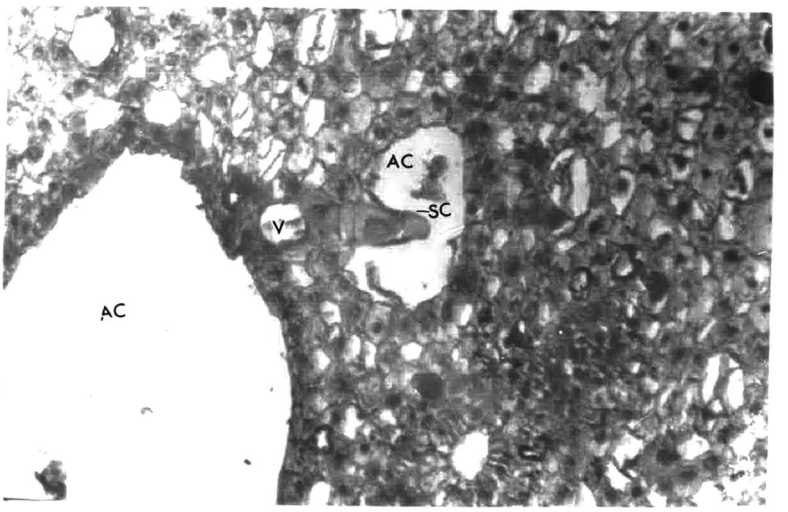

Fig. 3. Transverse section through petiole showing enlarging sclereid with vacuole; AC-air-canal, SC-sclereid, V-vacuole. X100.

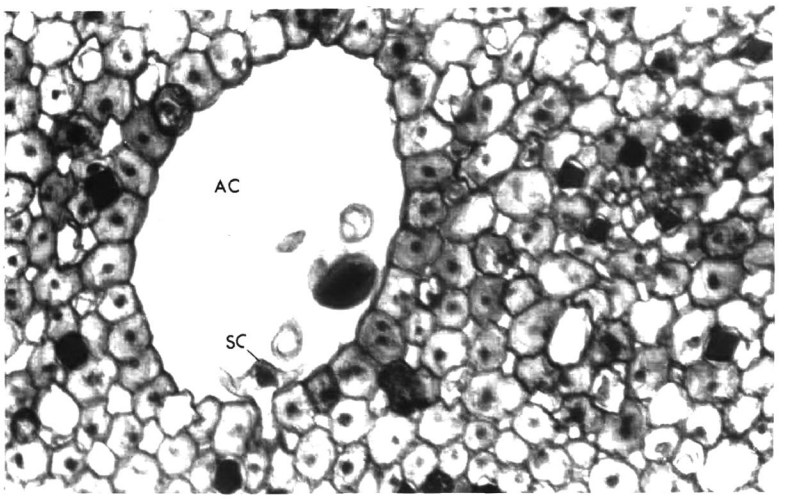

Fig. 4. Transverse section through petiole showing young sclereid in a more advanced stage of development; nucleus has migrated to tip; SC-sclereid, AC-air-canal. X100.

ontogeny by Arzee (1953b) in Olea.

As the initial develops, expansion may become strongly localized, occurring in from one to several directions (figs. 5, 6, 7). The expansions normally extend into adjacent associated air-canals (figs. 8, 9, 10). When the expansion takes place into two air-canals simultaneously a double bipolar (H-shaped) sclereid is formed (figs. 10, 11). Expansion in a third direction, into another canal or space, or into several spaces, results in a stellate sclereid (fig. 12).

Additional structural changes are associated with further sclereid development. Crystals, presumably of calcium exalate, begin to appear on the walls. At this stage of development, the walls are still primary. Secondary wall deposition is initiated later, appearing first in the older central region of the young sclereid, and progressing towards the tips of the expanding arms.

Lamina Sclereids

Two principal types of sclereids are found in leaves of Nymphaea odorata. In the midrib and spongy region, the stellate type occurs (fig. 13), and in the mesophyll, the I-shaped type is present (figs. 14, 15).

In the midrib of the lamina, sclereids are noticeable after the sclereids in the petiole have begun to form. At this stage of development, transections of the midrib reveal five, somewhat elliptical, vascular bundles--four of which are vertically oriented, and one horizontally oriented. Proceeding from upper to lower epidermis, in the central region of the petiole, the bundles occur in the following sequence: (1) a small collateral bundle, (2) a larger collateral bundle, (3) a bundle oriented at right

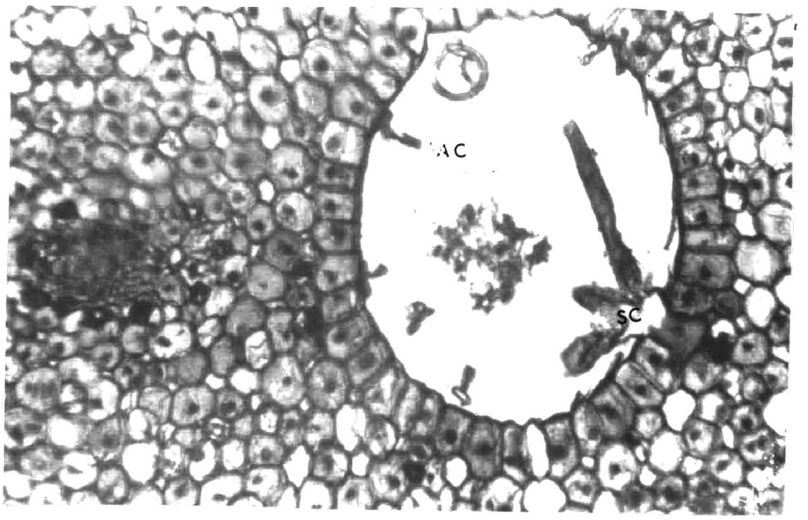

Fig. 5. Transverse section through petiole showing sclereid branching into air-canal; AC-air-canal, SC-sclereid. X100.

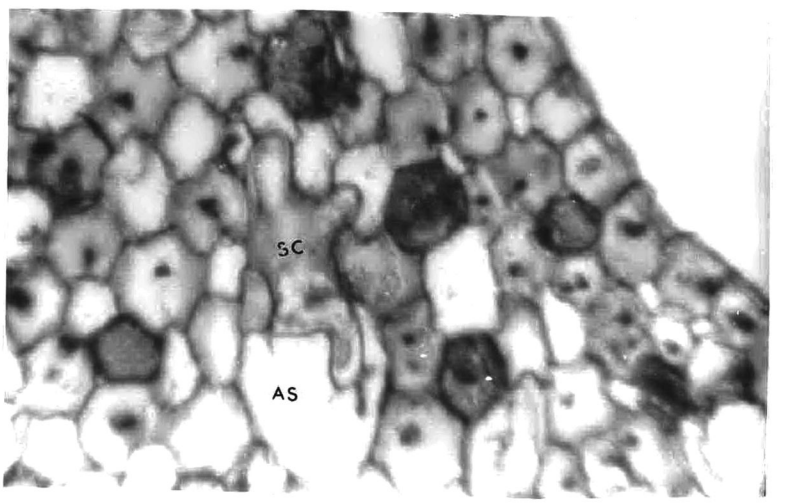

Fig. 6. Transverse section through petiole showing sclereid branching into air space; SC-sclereid, AS-air space. X400.

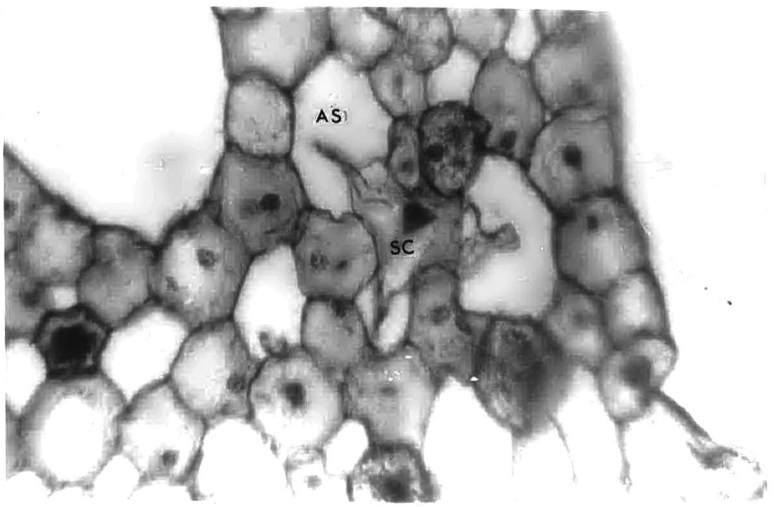

Fig. 7. Transverse section through petiole showing sclereid branching into three air spaces; SC-sclereid, AS-air space. X400.

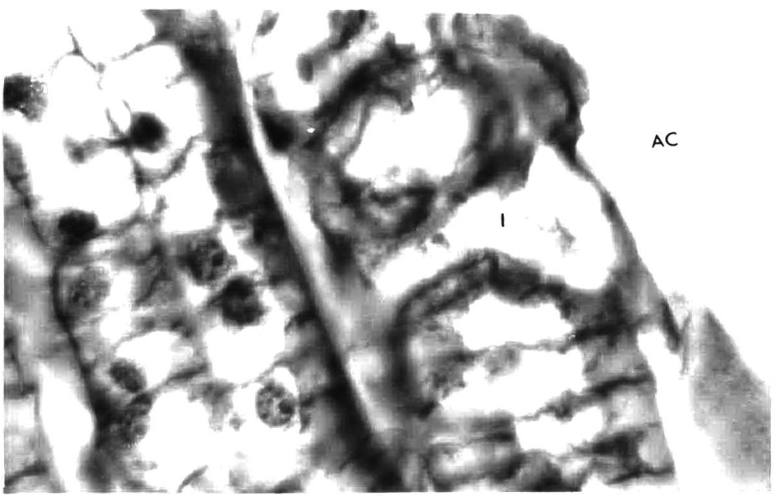

Fig. 8. Longitudinal section through petiole showing enlargement of sclereid initial in direction of an air-canal; I-initial, AC-air-canal. X970.

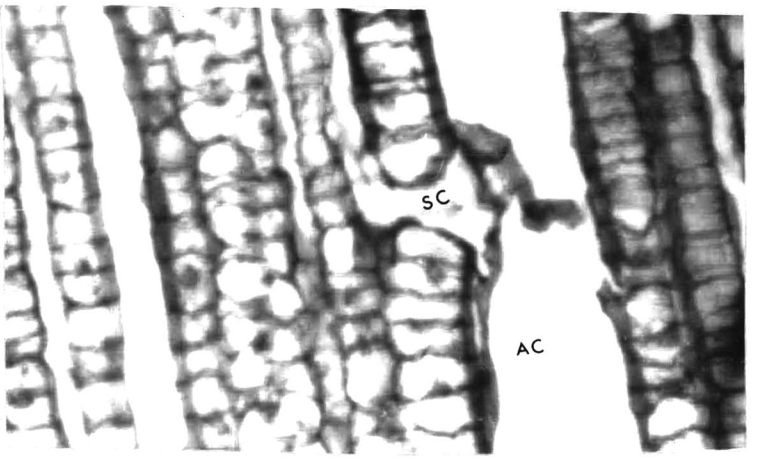

Fig. 9. Longitudinal section through section of petiole showing sclereid in a more advanced stage of development; SC-sclereid, AC-air-canal. X970.

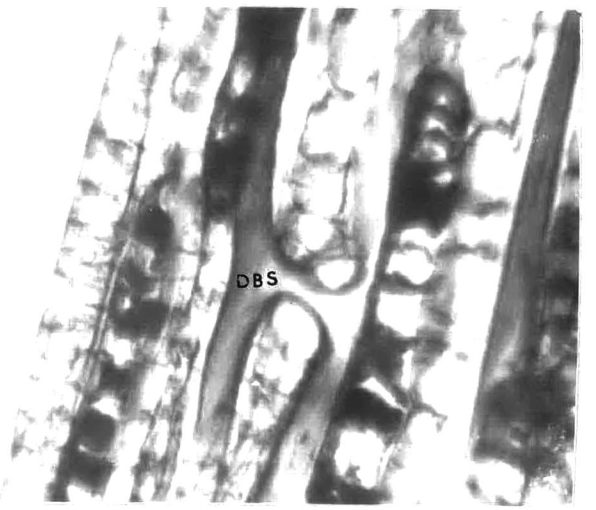

Fig. 10. Longitudinal section through petiole showing double bipolar sclereid; DBS-double bipolar sclereid. X100.

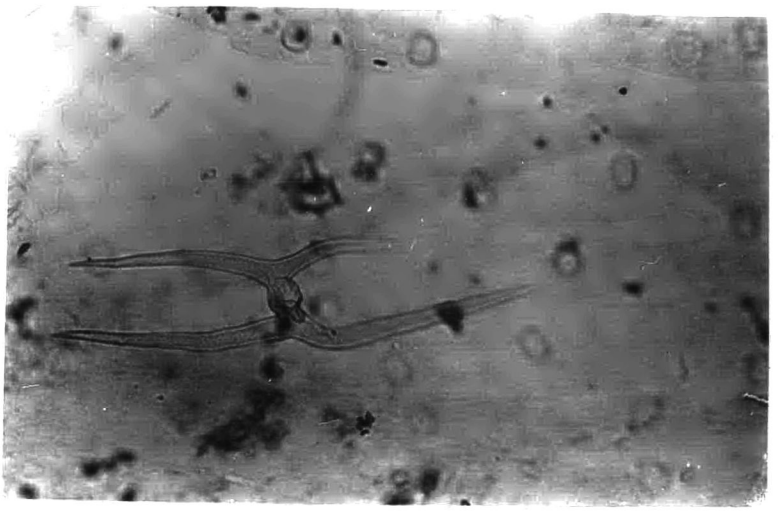

Fig. 11. Portion of cleared leaf showing double bipolar sclereid. X100.

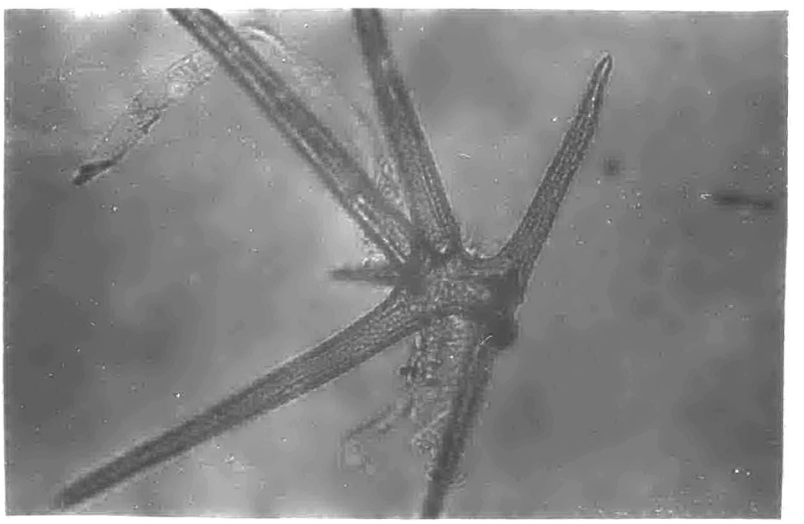

Fig. 12. Stellate sclereid from macerated leaf petiole. X100.

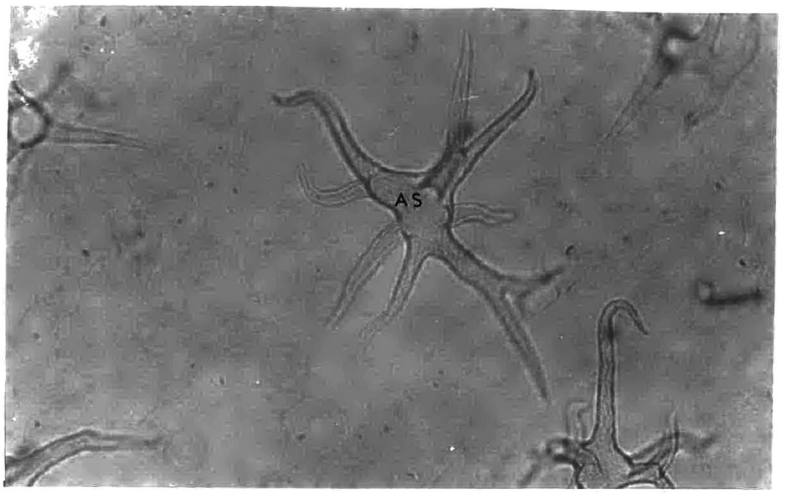

Fig. 13. Portion of cleared leaf showing astrosclereid; AS-astrosclereid. X100.

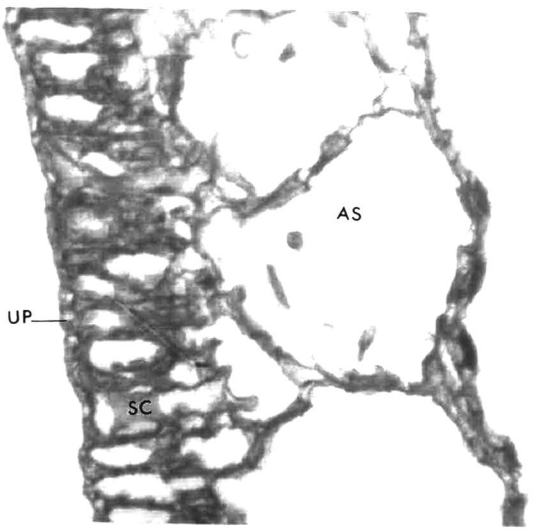

Fig. 14. Transverse section through leaf lamina showing young branching sclereid in palisade region; SC-sclereid, AS-air space, UP-upper epidermis. X100.

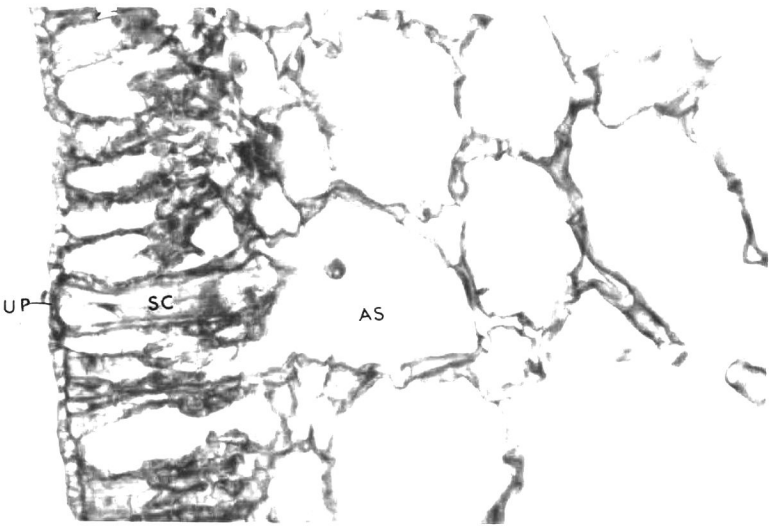

Fig. 15. Transverse section through leaf lamina showing elongating sclereid in further stage of development; UP-upper epidermis, SC-sclereid, AS-air space. X100.

angles to the axis of the other bundles, (4) a bundle with three patches of phloem—one above and one to each side of the xylem, and (5) a collateral bundle with xylem uppermost.

The basal region of the midrib is the place in the lamina where sclereid initials first appear. Vascular bundle formation appears to precede the appearance of these sclereid initials. Air-canals, like those found in the petiole, are absent in the lamina. Intercellular spaces are present in the lamina and are evidently associated with air-canals in the petiole.

In early leaf development, the lamina is essentially as long as the petiole. When the lamina reaches a length of about 10 mm, all of the tissue still appears to be meristematic. At this stage, the young lamina is unexpanded and is tightly rolled inwardly on both sides of the midrib. Cross sections of the lamina reveal many differentiating vascular bundles (fig. 16). As lamina development progresses, air spaces appear in the spongy region of the mesophyll. Concomitant with air space formation, is the appearance of sclereid initials (fig. 17).

According to Gaudet (1960), the initials are usually exposed on two sides to the large intercellular spaces. The observations reported here are in agreement with Gaudet's report. At first, expansion takes place in two directions, similar to the pattern described for sclereid development in petioles. Branching may subsequently take place in other directions resulting in a stellately branched sclereid. Sclereids develop in the spongy region before the palisade parenchyma fully differentiates. With further leaf growth, the palisade region differentiates, and some of the cells in this region give rise to sclereid initials. With further growth, the sclereid becomes vertically oriented. The upper tip develops a slight lateral

Fig. 16. Transverse section through rolled leaf lamina showing numerous air spaces in the developing spongy region and showing differentiating vascular bundle; AS-air space, VB-vascular bundle. X100.

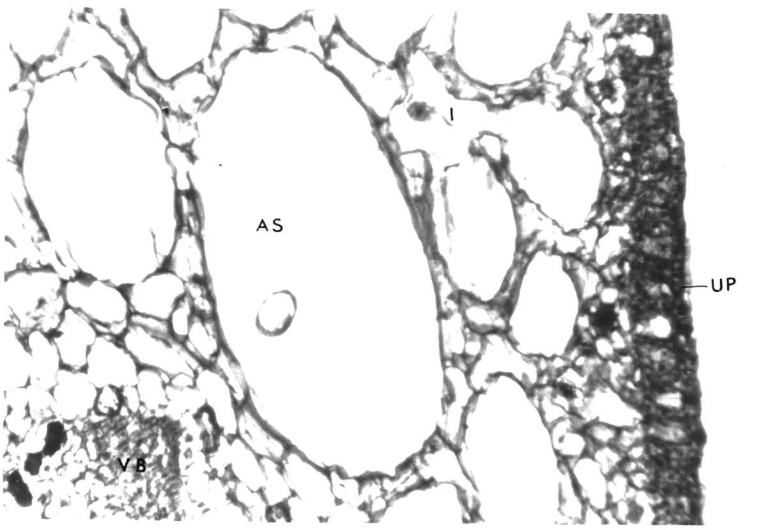

Fig. 17. Transverse section through leaf lamina showing sclereid initial developing in spongy region; I-sclereid initial, AS-air space, UP-upper epidermis, VB-vascular bundle. X100.

expansion, thus forming a somewhat I-shaped sclereid (note fig. 14). The lower part of this developing sclereid branches into the intercellular spaces of the spongy region.

Sclereid Development and Distribution

It was mentioned above that nuclear enlargement represented the first indication of sclereid initiation. The nuclei of the initials in both the petiole and in the lamina measure 19.8 microns which is twice the size of the nuclei of the neighboring cells in the spongy region and in the petiole. These latter nuclei measure 9.9 microns in both regions.

Sclereid initials in the lamina, at first, appear to be larger than those in the petiole (compare figs. 1 and 17). As the initials expand in some regions of the leaf, prismatic crystals appear on the walls. The first indication of crystal formation occurs after the initial has begun to branch. At this stage of development the walls of the initials are still primary. These crystals appear to be encrusted on the outer surface of the primary wall. They are noticed even before the formation of the secondary wall. This suggests that the crystals are not located between the primary and the secondary walls as mentioned by Gaudet (1960) in referring to the work of Gurtler and Frey-Wyssling. The chemical material may be secreted through the wall and accumulate as crystals on the outer surface of the primary wall.

On isolated sclereids from macerated leaves, crystals are not visible on the outside of the primary wall; instead impressions seem to be present, indicating the former site of crystals. It is possible that, if crystals are on the outer surface of the primary wall, the macerating fluid may have dislodged or dissolved them.

Sclereid development, in leaves of Nymphaea odorata, occurs centrifugally from the midrib towards the edge of the leaf lamina and acropetally from the base of the petiole. At first, in cleared leaves, sclereids appear only in the central region of the leaf lamina near the midrib, and in major veins branching from the midrib. As development proceeds, sclereids become scattered over the entire leaf except the outer areas of the lamina.

Observation of cleared leaves indicate that sclereids are present in young unrolled leaves measuring 7 mm to 18 mm in length. It appears that, for the most part, the greater the leaf length, the more numerous are the sclereids.

CHAPTER V

SUMMARY

Sclereid ontogeny and sclereid morphology have been studied in leaves of the water lily, Nymphaea odorata. For the ontogenetic studies, very young petioles measuring from 6-18 mm and lamina of leaves ranging from 5-15 mm were prepared for histological observations. Portions of young and older leaves were cleared for observations on the morphology of mature sclereids.

Two main types of sclereids have been found in leaves of Nymphaea odorata — trichosclereids and astrosclereids. Trichosclereids were found to occur in the petiole and developed from cells bordering air-canals and from cells associated with air spaces. There is some evidence that sclereid development may occur in the ground tissue of the petiole away from associated air spaces.

Astrosclereids were found to be the predominant type occurring in the leaf lamina. In the spongy region, the sclereids branch into the intercellular spaces and abut upon each other. Sclereids in the palisade region differed greatly from those in the spongy region by being I-shaped in form. Sclereids from the palisade region were elongate and sent branches into the spongy region.

In all cases observed, the first clear indication of sclereid development is nuclear enlargement. As development continues, there is pronounced

vacuolation of the initial, the appearance of prismatic crystals on the primary wall, and subsequent secondary wall formation.

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